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The embryo-sac of *Pandanus*

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(WITH PLATES 16 AND 17)

Among the interesting problems which are engaging the attention of botanists at the present time is the study of certain anomalies in the structure of the embryo-sac of the angiosperms, to which, of late, attention has been called. The stereotyped character of the embryo-sac structures was long taken for granted; but it is becoming more and more evident that the departures from the type are more numerous and more marked than has generally been supposed. Further discoveries in this direction are therefore naturally of interest.

As the Pandanaceae are considered to be among the lowest of the Monocotyledons, it was thought that they might show some deviations from the ordinary type of embryo-sac and a special effort was made to obtain material during a visit to the East Indies made in 1906. While in Java in the spring of 1906, I had an opportunity of collecting material of several species of *Pandanus*, and an examination of this made it evident that the embryo-sac in this genus differs very markedly from that known in any other plant. A preliminary note * was published giving the most important facts. The present paper treats in detail the development of the embryo-sac up to what is probably the stage of fertilization, but unfortunately none of the material was old enough to show the fertilization and the early stages of endosperm formation or the development of the embryo. The results, however, seemed important enough to warrant publication and are set forth at length in the following pages.

The most marked departure from the typical embryo-sac that has yet been observed is that of *Peperomia*, in which the writer first demonstrated that there were normally sixteen nuclei instead of the eight characteristic of the typical embryo-sac.† Through

* CAMPBELL, D. H. The embryo-sac of *Pandanus*. Preliminary note. Ann. Bot. 22: 330. 1908.

† CAMPBELL, D. H. The embryo-sac of *Peperomia*. Ann. Bot. 15: 103-118. pl. 6. 1901.

the work of Johnson * and Brown † other species have been investigated which agree in their essential structures with *P. pellucida*, the species in which the original discovery was made. A sixteen-nucleate stage was found by Schnegg ‡ to occur sometimes in *Gunnera*, and Ernst § has recently shown that this is regularly the case in *G. macrophylla*. In the latter, however, the embryo-sac in its mature condition resembles more nearly that of the typical angiosperms than does that of *Peperomia*. In a preliminary announcement Stephens || has stated that in several genera of the South African family Penaeaceae the embryo-sac regularly contains sixteen nuclei, but the details have not yet been published. Certain Araceae also show certain marked deviations from the usual type, but these are not constant and their meaning is still obscure.¶ Shattuck ** reports in *Ulmus americana* a condition intermediate between *Peperomia* and the typical angiosperms. More or less marked deviations from the type have also been observed in *Juglans* †† and certain Liliaceae, but these are less striking than in *Peperomia* and *Gunnera*. In a number of plants representing widely separated families peculiarities in the antipodal cells have been observed, which, in some cases at least, point to an increase in the vegetative tissues of the gametophyte. There is often a greater or less enlargement of the antipodal cells which may be accompanied by an increase in their number. This usually follows after

* JOHNSON, D. S. On the endosperm and embryo of *Peperomia pellucida*. Bot. Gaz. 30: 1-11. pl. 1. 1900.

— On the development of certain Piperaceae. Bot. Gaz. 34: 321-340. pl. 9, 10. 1902.

† BROWN, W. H. The nature of the embryo-sac of *Peperomia*. Bot. Gaz. 46: 445-460. pl. 31-33. 1908.

‡ SCHNEGG, H. Beiträge zur Kenntniss der Gattung *Gunnera*. Flora 90: 161-208. 1902.

§ ERNST, A. Zur Phylogenie des Embryosackes der Angiospermen. Ber. Deuts. Bot. Gesells. 26a: 419-438. pl. 7. 1908.

|| STEPHENS, E. L. A preliminary note on the embryo-sac of certain Penaeaceae. Ann. Bot. 22: 329. 1908.

¶ CAMPBELL, D. H. Studies on the Araceae. The embryo-sac and the embryo of *Aglaonema* and *Spathicarpa*. Ann. Bot. 17: 665-687. pl. 30-32. 1903.

— Studies on Araceae, III. Ann. Bot. 19: 329-349. pl. 14-17. 1905.

** SHATTUCK, C. H. A morphological study of *Ulmus americana*. Bot. Gaz. 40: 209-223. 1905.

†† NAWASCHIN, S. Ein neues Beispiel der Chalazogamie. Bot. Centralbl. 63: 353-357. 1895.

fertilization, *e. g.*, *Sparganium* and several Araceae, or this increase in number may take place before fertilization, as in certain grasses.* Many Ranunculaceae are also characterized by a large development of the antipodal cells, which may become multi-nucleate, but according to Mottier † the nuclear division is amitotic and is presumably a secondary condition. The increase in the number of antipodals in the Compositae and some other sympetalous forms is considered by Coulter ‡ to be a secondary condition associated or involved in the formation of a haustorial organ.

In *Pandanus* there is an increased number of nuclei, *i. e.*, 14 instead of 8, and to judge from the most advanced stages that were met with, there is a failure to develop the definite structures of the typical embryo-sac. It is possible an examination of older material may show a development of further embryo-sac structures before fertilization takes place.

The Pandanaceae, comprising the genera *Pandanus* and *Freycinetia*, are confined entirely to the old world, being especially abundant in the Malayan region, where they constitute a very striking feature of the flora. One species of each genus extends to the Hawaiian Islands. The Pandanaceae are usually considered to be among the lowest of the monocotyledons, and the present work was undertaken to see whether the embryo-sac structures showed any deviations from the ordinary angiospermous type which might be interpreted as of primitive nature. The results, as we shall see, indicate that the embryo-sac in *Pandanus*, at least, normally differs very much from that of the typical angiosperms, and that the differences indicate a less specialized condition, approximating the very generalized type of *Peperomia*.

The material upon which the following account is based was collected in Buitenzorg during my stay at the botanic gardens there from March to June, 1906. The collection of Pandanaceae in the Buitenzorg Gardens is without much question the richest in the world, and while there I collected all of the suitable material there

* CANNON, W. A. A morphological study of the flower and embryo of the wild oat, *Avena fatua* L. Proc. Calif. Acad. III. 1: 329-364. *pl.* 49-53. 1900.

† MOTTIER, D. M. Contributions to the embryology of the Ranunculaceae. Bot. Gaz. 20: 241-248, 296-304. *pl.* 17-20. 1895.

‡ COULTER, J. M., & CHAMBERLAIN, C. J. Morphology of angiosperms, page 102. 1903.

was available. I must here express my deep appreciation of the great help afforded me by Professor Treub and the Director of the Garden, Mr. H. J. Wigmann, who gave me every assistance in procuring specimens. *Pandanus* offers special difficulties in securing material, as most of the species do not flower freely, and even under the extraordinarily favorable conditions at Buitenzorg only a small number of species flowered during my three months' stay.

Many of the specimens growing in the garden at Buitenzorg are still unnamed, as the limits of the species are still very poorly understood, and the large genus *Pandanus* seems to be very much in need of a thorough revision. Three of the species collected, however, were named, two of them being comparatively small species: *P. Artocarpus* Griff., and *P. affinis* Kurz. The other was the large, wide-spread species *P. odoratissimus* L. f., which extends as far as the Hawaiian Islands. The material was fixed in several ways: one per cent. chromic acid, weak Flemming's solution, and acetic alcohol. All of the methods proved satisfactory. The younger flowers offer no difficulties in the way of fixation, but in the later stages the outer part of the carpels becomes hard and this must be cut away so as to expose the softer tissues surrounding the ovule. Owing to the small number of inflorescences that were available, it was impossible to obtain any of the stages subsequent to fertilization, but it is hoped that material may be procured which will supplement the account of the earlier stages which is given here.

All of the Pandanaceae are dioecious. The pistillate flowers in *Pandanus* are in dense heads, which in the smaller species are borne several together at the end of a short branch, each head being subtended by a conspicuous bract. In the larger species, where the head of fruit may be as big as a large pineapple, the heads are solitary and enveloped in a large number of bracts. The Pandanaceae have been placed close to the Sparganiaceae, and the heads of flowers, as well as the spiky fruit look very much indeed like those of *Sparganium*. In the smaller species; e. g., *P. Artocarpus* (FIG. 1) the flower consists of a single carpel, which, in form as well as in the position of the ovule, resembles that of *Sparganium simplex*, but the conspicuous scale-like bracts that surround the carpel in *Sparganium* are absent. In the large species like *P.*

odoratissimus several carpels are more or less completely coherent, and may be compared to the compound pistils of *Sparganium eurycarpum* or *S. Greenii*. Each carpel contains a solitary anatropous ovule attached to one side of the ovarian cavity very near its base. The placenta and the base of the funiculus bear thickly placed glandular hairs. These are probably concerned with the conduction of the pollen-tube to the micropyle.

The ovule presents no notable peculiarities in its form. There are two integuments, of which the inner one in the younger ovules projects somewhat beyond the outer one, which is not very strongly developed but finally reaches the level of the inner integument. On the inner side of the ovule the outer integument is completely adherent to the funiculus (FIG. 2). The embryo-sac is separated from the apex of the nucellus by several layers of cells.

The nucellus is more or less pointed at the top, and there is a very sharply defined epidermis consisting of a single layer of cells except at the apex of the nucellus, where periclinal divisions occur, so that there may be three or four layers of cells in this region. This formation of periclinal walls, combined with an elongation of the cells, produces the more or less prominent beak which is usually present. FIGURE 3 shows a median longitudinal section of one of the youngest ovules that was found. This was from *P. affinis*, which differs slightly from the other species examined in the form of the embryo-sac mother-cell, as well as in the greater thickness of the epidermis at the apex of the nucellus. As the older stages of this species were not available, it is impossible to say how they would compare with the corresponding stages in the other two species that were studied.

The embryo-sac mother-cell at this stage is undivided, but it is easily recognized by its dense contents and its very conspicuous nucleus. In section it appears almost triangular in form with a pointed base and broad apex. Between it and the epidermis is a group of cells, probably tapetal or "parietal" cells, and, to judge from the form and position of the group, they arise from the division of a single cell which presumably is a sister-cell of the embryo-sac mother-cell.

DEVELOPMENT OF THE EMBRYO-SAC IN PANDANUS ARTOCARPUS

The earliest stages of the embryo-sac found in *P. Artocarpus* (FIGS. 4-6) were somewhat more advanced than that described for *P. affinis*. The mother-cell is more nearly cylindrical and the base is truncate and sometimes almost as broad as the apex, and in such cases it forms the lowermost of a single series of cells, or at least such is the appearance in a longitudinal section. (See FIG. 6.) More frequently the upper end of the mother-cell is broader and two series of parietal cells appear in longitudinal sections. These two series probably arise from a median division of the tapetal or "parietal" cell, and by subsequent periclinal divisions give rise to about three layers of cells between the mother-cell and the epidermal layer at the apex of the nucellus. The sporogenous cell divides by transverse division into two cells, of which the lower is the larger and becomes the embryo-sac. The upper cell, x , divides again into two by a vertical wall and these two small cells persist with little change for a long time, but finally disintegrate and are visible only as two small, darkly stained, shrunken bodies lying above the apex of the embryo-sac. In these early stages *Pandanus* conforms closely to the ordinary angiosperms, except for the vertical division in the upper sporogenous cell, and differs from *Peperomia* and *Gunnera* in the formation of three cells which may be interpreted as megaspores, instead of having the sporogenous cell develop directly into the embryo-sac without preliminary division.

Unfortunately no nuclear divisions were found, although these were carefully looked for, and this was true also for the later stages in the development of the sac. This was perhaps due to the fact that the material was all collected at about the same time of the day. The collectors brought the material in usually at about eight o'clock in the morning, and apparently the nuclear divisions occur at some other period of the day.

It is probable, as in other forms that have been critically studied, that the first reduction division occurs when the spore mother-cell first divides.

The youngest stage met with in *P. Artocarpus* is shown in FIGURE 6. The young embryo-sac is easily recognizable, the cytoplasm being noticeably more densely granular than that of the

adjoining cells of the nucellus, and the nucleus is somewhat larger. The nucleolus in this case lay in the cytoplasm near the nucleus, but this was doubtless the result of the nucleus having been sectioned and the nucleolus accidentally displaced in mounting the section. At this stage the embryo-sac had enlarged very little, being only slightly broader than the sister-cell above it, which in this instance was rather larger than usual, although there is a good deal of difference in different cases in this particular. FIGURE 4 shows a slightly more advanced stage. The upper sporogenous cell is smaller and the nucleus less conspicuous than usual. The appearance of the transverse division in the upper cell is probably due to a shrinkage of the protoplasm, and this probably also accounts for the very small and poorly defined nucleus. The cytoplasm of the young embryo-sac is vacuolate, and the conspicuous nucleus with its single nucleolus occupies the center of the cell. A slightly older stage is shown in FIGURE 5. The vacuoles have become more numerous but less definite and the nucleus is noticeably larger. The sister-cell has divided longitudinally instead of transversely as is the case in most angiosperms. While this longitudinal division is rather unusual, it has been observed in a number of other forms, both in monocotyledons and dicotyledons.*

The young embryo-sac rapidly increases in size and the nucleus divides, one of the daughter nuclei moving to the upper end of the sac, the other to the chalazal end. The two nuclei are quite similar in appearance (FIG. 8), and the cytoplasm still occupies the whole cavity of the sac, although there are numerous large vacuoles. The large vacuoles in the center of the sac finally unite, and by the time the second mitosis is complete (FIG. 9) a single large vacuole occupies the greater part of the sac and the cytoplasm is mainly confined to the ends of the sac, there being only a thin layer lining the lateral walls.

Up to this point *Pandanus* agrees exactly with the typical angiosperms, but the subsequent history of the embryo-sac is decidedly different. The two nuclei at the micropylar end of the sac undergo no further division, and in the oldest stages that were found, two nuclei only, unchanged except for an increase in size,

* COULTER, J. M., & CHAMBERLAIN, C. J. Morphology of angiosperms, pages 75, 76. 1903.

were invariably present. The history of the chalazal nuclei is different. FIGURE 10 shows a stage with four chalazal nuclei, of which three appear in the section shown. The cytoplasm has increased noticeably in amount, and this becomes still more marked in the later stages. Owing to the failure to secure division stages, the sequence of nuclear divisions by which the number of the chalazal nuclei is increased cannot be determined. Several cases, one of which is shown in FIGURE 11, show six chalazal nuclei, but whether there is any rule as to which of the four nuclei of the preceding stage divide and which remain undivided must for the present remain in doubt, and the same is true of the subsequent division by which the number of chalazal nuclei increases to eight and finally to twelve. There seem to be regularly twelve chalazal nuclei in the older stages, although it is possible that in some cases there may be only ten.

There is more or less variation in the form of the lower end of the embryo-sac. It is sometimes pointed (FIGS. 9, 12), or it may be broad and rounded. Not infrequently one or more of the cells of the nucellus push up into it, so that the lower end of the sac is divided into short, sac-like extensions (FIG. 11, *a*).

In the most advanced stages that were secured, the embryo-sac had increased materially in size. At the micropylar end, which is narrower than the chalazal end, there are two nuclei, while at the chalazal end there are twelve nuclei embedded in a large mass of densely granular cytoplasm containing several conspicuous vacuoles (FIGS. 14-16). A similar but smaller mass of cytoplasmic material occupies the micropylar end, and the large central vacuole is bounded laterally by a rather thick layer of cytoplasm, which, however, contains no nuclei. In most cases observed there was no apparent differentiation of the micropylar cytoplasm, but in a very few instances, the most conspicuous of which is shown in FIGURE 14, there was a slight indication of what looked like the separation of an egg-cell and synergid, but this was very vague, and the limits of the egg, if such it was, were very poorly defined. At this stage, the two cells lying above the embryo-sac are still visible, but are evidently disintegrating.

Corresponding to the enlargement of the embryo-sac is a marked increase in the size of the nuclei, which at the same time

show a distinct reticulum, while in the younger sac the contents of the nucleus appear more compact and uniformly granular. The nucleolus also increases much in size and becomes very conspicuous. In *P. Artocarpus* the increase in size is more marked in the chalazal nuclei than in the micropylar ones, which are noticeably smaller. In the most advanced stages the arrangement of the nuclei is very similar to that in *Peperomia hispidula* described by Johnson, except that there are fourteen instead of twelve chalazal nuclei in the latter, and these ultimately fuse into one enormous endosperm nucleus. No indications of any nuclear fusions were met with in any of the embryo-sacs of *Pandanus*, and the final history of these large chalazal nuclei must for the present remain undecided.

Pandanus odoratissimus was also examined for comparison with *P. Artocarpus*, from which it was found to differ only in some minor particulars. This species has very large fruits with the carpels united into groups, forming more or less complete compound pistils, but the union of the carpels is a very loose one. The upper part of the carpels soon becomes very hard and woody, but the base remains succulent for some time and is easily sectioned. The inflorescences from which the preparations were made were about 6 centimeters in diameter, and it was supposed that these carpels had already been fertilized. It was therefore hoped that the later stages of the sac would be obtained, but examination showed that in spite of the large size of the carpels, which were nearly 2 centimeters in length, the ovules were little further advanced and not noticeably larger than those in the apparently much younger and smaller flowers of *P. Artocarpus*. An examination of the stigma showed the presence of pollen-spores, some of which were still ungerminated, while others had emitted the pollen-tubes. None of the ovules that were examined, however, showed the presence of the pollen-tube, and the question must still remain open whether the sac undergoes any further changes before the actual fertilization occurs. In *P. odoratissimus* the two micropylar nuclei are quite as large as those of the chalazal region (FIG. 21), and the largest sac found in this species was slightly larger than that of apparently the same age in *P. Artocarpus*. In *P. odoratissimus* a few nuclei were seen which con-

tained two nucleoli, and one of these nuclei looked as if it possibly might have been the result of the fusion of two.

Owing to the similarity in the appearance of the nuclei of the nucellus-cells adjacent to the young embryo-sac and those of the sac itself, the former may sometimes be mistaken for those belonging to the embryo-sac, but in the later stages the much greater size of the embryo-sac nuclei makes it very easy to recognize them. However, a structure is occasionally met with that is at present not quite clear. There is sometimes found a small cell (FIGS. 13, 15, *y*) apparently cut off from the side of the embryo-sac, recalling the small cells of similar appearance cut off from the sac in *Peperomia*. The contents of these cells are densely granular like those of the embryo-sac, but the nuclei are usually small, and I am inclined to believe at present that these cells really belong to the nucellus and have pushed into the cavity of the embryo-sac somewhat in the same fashion as those already mentioned as sometimes pushing up from below. The occasional occurrence of a small nucleus apparently free in the cytoplasm of the sac, and differing in appearance from the other nuclei, suggests that possibly the wall of this intruding cell may have become resorbed, discharging the nucleus into the embryo-sac. These points cannot be settled, however, until the history of the nuclear division is known.

All of the cells surrounding the embryo-sac differ more or less from the outer nucellar tissue, having more watery contents and sometimes rather larger nuclei. They are probably concerned to some degree with the nutrition of the embryo-sac, and sometimes this central mass of tissue suggests a mass of sporogenous cells; and it is not impossible that it really may represent a mass of sporogenous tissue of which only one cell gives rise to the spores.

Various attempts have been made to explain, as modifications of the ordinary angiospermous embryo-sac, the peculiar conditions found in *Peperomia* and the other forms with a double number of nuclei. Johnson and Brown both believe that the embryo-sac in *Peperomia* represents four megaspores and not a single one; and Coulter* in a recent paper maintains that the embryo-sac

* COULTER, J. M. Relation of megaspores to embryo-sacs in angiosperms. Bot. Gaz. 45: 361-366. 1908.

mother-cell in all cases where there is no previous division is to be regarded not as a single megaspore, but as an aggregate of four. He thinks that the nature of the reduction division which precedes the formation of the embryo-sac necessarily involves the production of four megaspores, or at least of four nuclei which represent them. It undoubtedly is true that so far as we know at present the reduction division cannot be omitted where fertilization is to occur, but as Brown has pointed out, it does not follow that the presence of the heterotypic division in a cell in the nucellus is alone sufficient ground for holding that such a cell is necessarily a megaspore mother-cell, inasmuch as the reduction division may occur at various points in the life history of a plant. He further points out the well-known fact that all stages of reduction in the sporogenous tissue may be traced from the ferns, where a single archesporial cell gives rise to a mass of tapetal cells and sporogenous tissue, to such angiosperms as *Lilium*, where the sporogenous cell at once forms a single megaspore. He says, "It does not seem reasonable to suppose that the division of the mother-cell into four megaspores may not also be left out and the mother-cell function directly as a megaspore. In this case the heterotypic division might be pushed forward and take place in the embryo-sac." The fact that the embryo-sac is actually, if not theoretically, part of the sporophytic structure, and the extensive reduction of the sporogenous tissue in the cells of the angiosperms in general make it somewhat rash to assume that the limits between sporophyte and gametophyte are as sharply drawn as they are in the pteridophytes.

Brown bases his opinion that the four nuclei in the young embryo-sac of *Peperomia* represent separate megaspores, upon the fact that in the two first nuclear divisions in *P. Sintenisii* transverse cell walls are formed, and in *P. pellucida* cell plates accompany the two first nuclear divisions. In the third division which results in the eight-nucleate stage, cell plates are wanting. But, as in the last division, by which the sixteen-nucleate stage arises, cell plates are formed, and, in the case of certain of the nuclei, cell walls also, this seems rather inadequate grounds for assuming that the embryo-sac represents four spores instead of a single one.

While the single megaspore in *Peperomia* and other similar

forms cannot probably be considered a primitive condition, this does not imply that the embryo-sac structures themselves may not be primitive in character. It is a significant fact that in the great majority of angiosperms the structures of the embryo-sac are not in the least affected by the number of divisions in the sporogenous tissue that precede the formation of the embryo-sac. The great variability in this respect shown by plants of near affinity, as in the often cited case of the Liliaceae, indicates that this is a matter of secondary importance. It certainly seems hardly likely that a structure made up of four megaspores should behave exactly like one derived from the germination of a single spore, and the generally accepted view that in such cases as *Peperomia* and *Lilium* the embryo-sac is a single megaspore formed without previous division from the mother-cell can hardly be admitted to have been disproved by these recent speculations. If we admit Coulter's views as to the compound nature of the embryo-sac of *Lilium*, we have still to explain why in *Peperomia*, where the course of development up to the beginning of the division of the embryo-sac is precisely the same as that of *Lilium*, there should later be such an extraordinary departure from the usual angiospermous type. And if the sixteen-nucleate embryo-sac of *Peperomia* is derived from the eight-nucleate one of a form like *Heckeria*, it remains to be explained why there are sixteen nuclei instead of eight, and why the subsequent history is so different. In the Liliaceae, where there is an entirely analogous condition of things, the structure of the mature embryo-sac is not in the least influenced by the fact that in some cases there is a division of the mother-cell and in other cases the division is suppressed, and it is hard to see why, in the case of *Peperomia*, the development of the embryo-sac directly without preliminary division should result in such remarkably different structures, unless we admit that the sixteen-nucleate stage is an earlier type of embryo-sac which has persisted in a few existing genera like *Peperomia* and *Gunnera*.

In *Gunnera macrophylla*, Ernst found the early development of the embryo-sac to be very much like that of *Peperomia*, but there was later developed a definite egg-apparatus of the normal type and a group of conspicuous antipodal cells. In *G. Hamiltonii*, according to Schnegg, these structures are much less evident. Ernst

believes that the single sporogenous cell in *Peperomia* and *Gunnera* is less primitive than the four cells found in so many angiosperms, but he does not believe that this suppression of the divisions is in any way associated with the peculiar characters of the embryo-sac itself. He thinks that the sixteen-nucleate embryo-sac should be recognized as an independent type not in any way derived from the ordinary eight-nucleate sac, and this we believe will really prove to be the case. Whether the ordinary eight-nucleate type has been derived from this sixteen-nucleate form may be questioned; but the condition in *Gunnera macrophylla*, which is almost intermediate in character between *Peperomia* and the ordinary angiospermous type, makes this not at all improbable. I believe we are justified in assuming that the condition in *Gunnera macrophylla* really does represent a condition nearly intermediate between the very primitive condition found in *Peperomia* and the familiar eight-nucleate sac of the typical angiosperms. The recent announcement of still another sixteen-nucleate sac in the Penaeaceae may be referred to again in this connection.

The peculiar embryo-sac of *Pandanus* cannot be explained on the theory of reduction from the typical eight-nucleate form. The embryo-sac in this case is one of three sporogenous cells, and even if we admit that it is composed of two megaspores, in one of these the nuclei are twelve in number instead of eight, while the other has only two. Of the forms hitherto described, *Peperomia hispida* most nearly resembles *Pandanus* in the arrangement of the nuclei in the embryo-sac.

SUMMARY OF RESULTS

1. The pistillate flower of *Pandanus* may consist of a single carpel, or of several loosely united carpels. Each carpel bears a single anatropous ovule.
2. The primary sporogenous cell is separated from the epidermis of the nucellus by several layers of parietal or tapetal cells, which are presumably derived from the division of a single tapetal cell.
3. The primary sporogenous cell divides transversely into two cells, of which the lower, and larger one, gives rise at once to the embryo-sac; the upper cell divides by an anticlinal wall again into two equal cells.

4. The first division in the embryo-sac results in two similar nuclei which occupy the poles of the sac.

5. The micropylar nucleus divides but once; usually no trace of a differentiation into egg-cell and synergid can be seen, but occasionally there seems to be a suggestion of such differentiation.

6. The primary chalazal nucleus divides repeatedly until twelve nuclei are formed; but the order of these nuclear divisions could not be determined, and the twelve resulting nuclei look very much alike.

7. The nuclei increase in size as the sac grows, but in no cases were there seen any indications of nuclear fusions. In the most advanced stages that were secured, all of the fourteen nuclei were quite separate.

8. In *P. Artocarpus* the two micropylar nuclei are somewhat smaller than the chalazal nuclei; in *P. odoratissimus* they are equal in size; otherwise the two species agree closely in the structure of the embryo-sac.

CONCLUSION

Until the fertilization stages can be examined, it will not be possible to state positively that the fourteen-nucleate stage really represents the condition of the sac at the time of fertilization, and the further history must be left for future investigation. Whether these investigations will reveal in the post-fertilization stages conditions approximating those in *Sparganium*, which in its floral structure resembles *Pandanus*, remains to be seen. *Sparganium* in the early development of the embryo-sac follows the usual course of development, and it is not until later that there occurs the extraordinary development of the antipodal cells. To judge from the conditions of the sac in the oldest stages that were met with, *Pandanus* differs very much from the typical angiosperms, and has its nearest analogue in *Peperomia hispidula*. The increased number of nuclei is evidently perfectly normal, and can hardly have been derived from the ordinary eight-nucleate type, nor can it be explained as a case of fusion of four megaspores, since only one of the megaspore divisions is suppressed. Probably the two micropylar nuclei represent a primitive egg-apparatus consisting of the egg and a single synergid, but what relation, if any, the twelve chalazal nuclei bear to the antipodal and polar nuclei of the ordinary embryo-sac it is useless

to conjecture until the further history is known. For the present the embryo-sac of *Pandanus* must be assumed to represent a new type with its nearest analogue in *Peperomia*, from which it differs in its marked polarity in its early stages, and the apparent absence of any nuclear fusions, as well as in the fact that there are only fourteen instead of sixteen free nuclei. It probably represents a less specialized and more ancient type of sac than the typical eight-nucleate one.

The recent work of Porsch on the embryo-sac has not been seen by the writer, and so it will not be possible to discuss here the views of that writer as to the homologies of the parts of the embryo-sac. From the references to his work by other investigators, it seems that he regards the egg-apparatus and antipodal cells of the ordinary embryo-sac as representing two archegonia. The condition of things in *Pandanus* would certainly lend no support to such a view. The accumulating discoveries of plants in which the double, or approximately double number of nuclei is normally present in the embryo-sac, makes it more and more likely that, as Ernst maintains, this really is a distinct type of sac not derived from the eight-nucleate one, but an independent and presumably more primitive type. It is to be expected that further researches will add to the number of these sixteen-nucleate sacs and may throw more light upon the characteristic eight-nucleate sac which distinguishes the great majority of angiosperms.

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Explanation of plates 16 and 17

PLATE 16

FIG. 3 refers to *Pandanus affinis* Kurz, the others all refer to *P. Artocarpus* Griff. Except Figs. 1, 2, the drawings were all made with a Leitz $\frac{1}{18}$ oil immersion, oc. 1. The drawings are reduced about one fourth in the reproduction.

FIG. 1. A single pistillate flower of *Pandanus Artocarpus*, $\times 4\frac{1}{2}$; *o*, the ovule.

FIG. 2. Section of a young ovule, $\times$ about 40; *m*, the embryo-sac.

FIG. 3. Longitudinal section of the nucellus of a very young ovule of *P. affinis*; the sporogenous cell is still undivided; the nuclei of the parietal cells are shown.

FIG. 4. Young embryo-sac, *m*, and the sister-cell, *x*.

FIG. 5. Upper part of the nucellus showing the young embryo-sac, *m*, the sister-cells, *x*, and the parietal cells lying above.

FIG. 6. A somewhat younger stage than the last; only a single row of parietal cells.

FIG. 7. A somewhat older stage; the embryo-sac contains two nuclei, only one of which shows in the section.

FIG. 8. Embryo-sac with two nuclei; only one of the sister-cells, *x*, shows in the section.

FIG. 9. Embryo-sac with four nuclei; only one of the micropylar nuclei is seen in the figure.

FIG. 10. Embryo-sac with two micropylar and four chalazal nuclei.

PLATE 17

FIGS. 17-21 refer to *Pandanus odoratissimus* L., the others to *P. Artocarpus*; all figures drawn with Leitz $\frac{1}{16}$ oil-immersion, oc. 1. Reduced one fourth in reproduction.

FIG. 11. Embryo-sac having six chalazal nuclei, four of which are shown in *a*; one of the nucellar cells has pushed up into the base of the embryo-sac; *b*, the micropylar end of the same sac, showing the two cells, *x*, above the sac.

FIG. 12. *a*. Micropylar end of an embryo-sac with eight chalazal nuclei; the remains of the two sterile "megaspores" are seen above the sac. *b*. Chalazal end of the same.

FIG. 13. Transverse section of the chalazal region of an embryo-sac with 8 chalazal nuclei. The small cell, *y*, probably belongs to the nucellus.

FIG. 14. Upper end of an embryo-sac in which there was an apparent slight differentiation into a synergid (?), *s*, and an egg-cell (?), *o*; one of the chalazal nuclei is seen.

FIG. 15. Chalazal end of the same sac; there were probably 12 chalazal nuclei; the small cell, *y*, probably belongs to the nucellus.

FIG. 16. Chalazal end of one of the largest sacs that was found—12 chalazal nuclei were present.

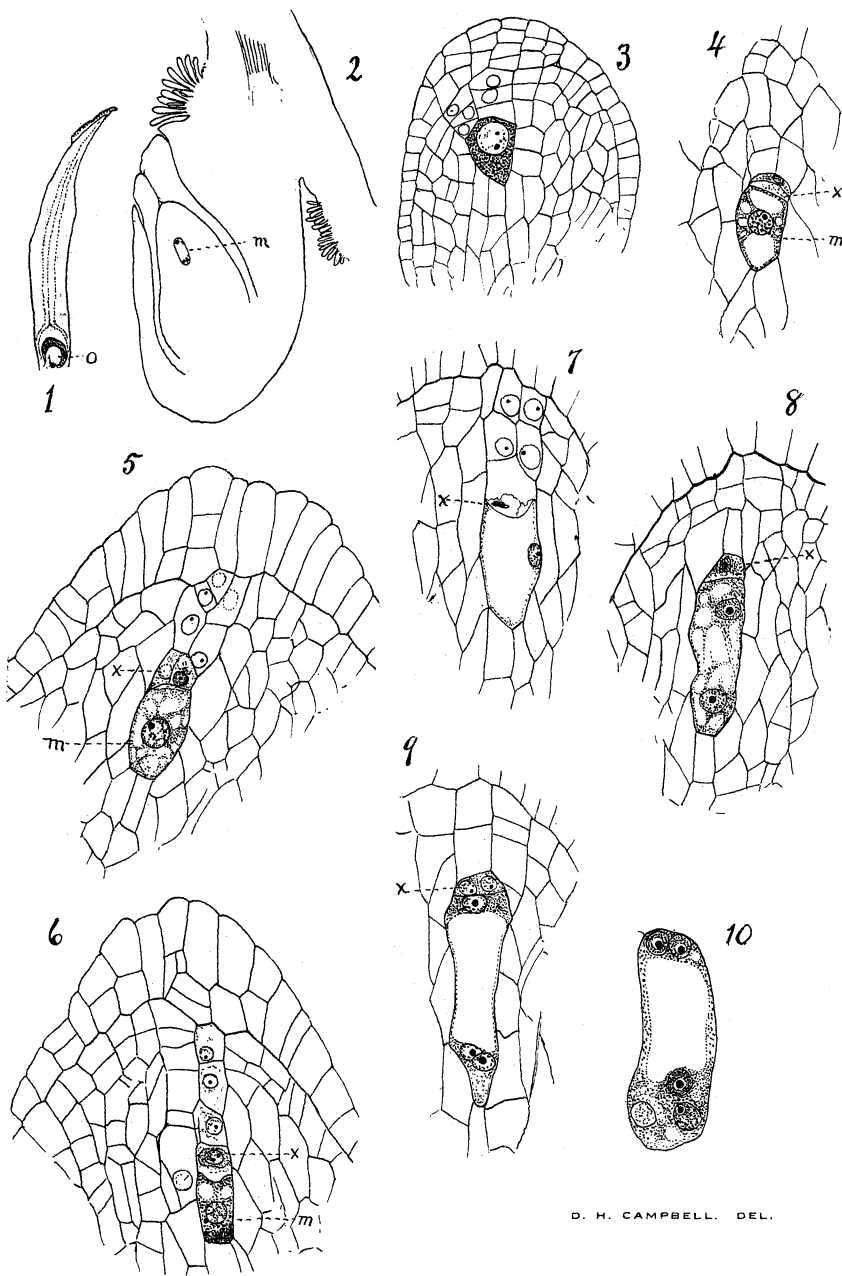
FIG. 17. Apex of the nucellus of a young ovule of *P. odoratissimus*; the embryo-sac had four nuclei.

FIG. 18. Micropylar end of the sac shown in Fig. 17; one of the two nuclei is shown.

FIG. 19. Mature (?) embryo-sac showing one of the two micropylar nuclei, and three of the twelve chalazal ones.

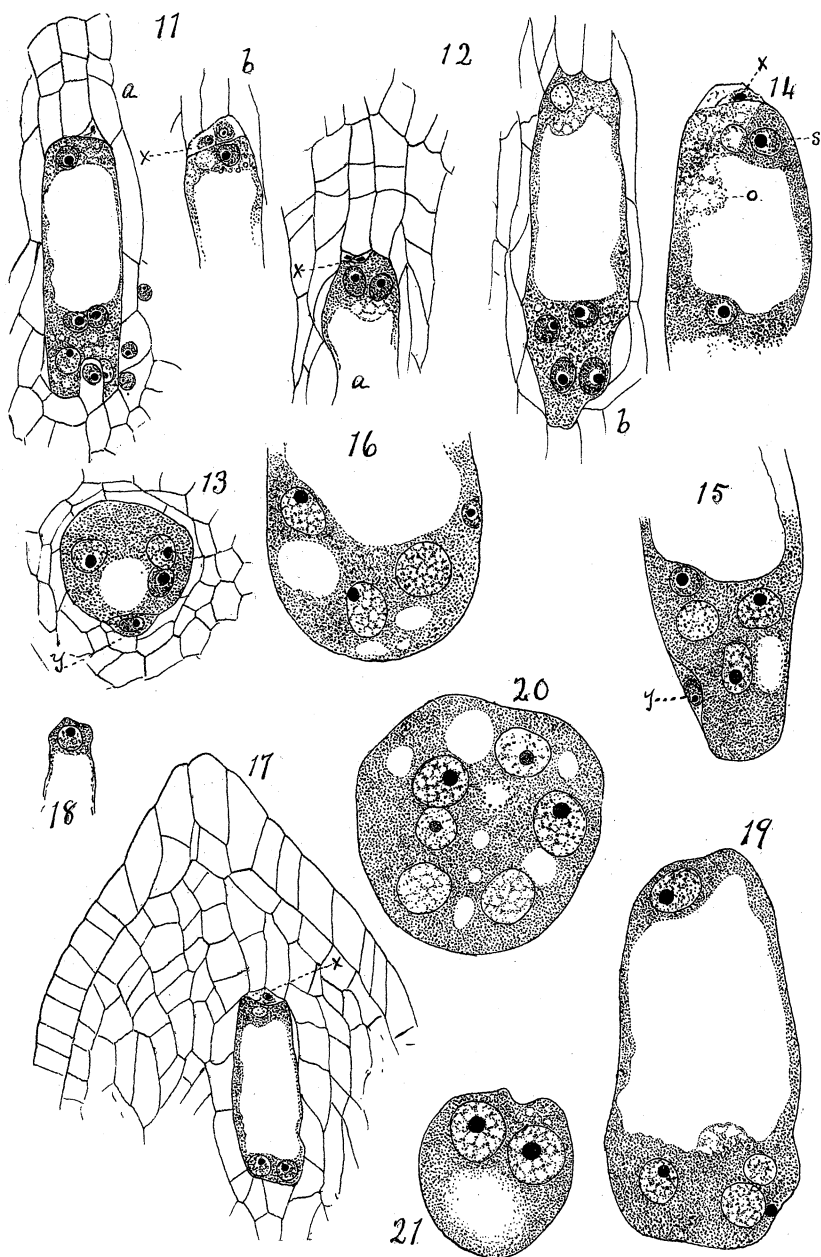
FIG. 20. Transverse section showing six of the twelve chalazal nuclei.

FIG. 21. The two micropylar nuclei from the same sac.



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